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Published:
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/042244 A3

(54) Title: IMMUNOGENIC MIMETICS OF MULTIMER PROTEINS WITH PROMISCUOUS T CELL EPITOPE INSERTS

(57) Abstract: The present invention relates to novel immunogenic variants of multimeric proteins such as immunogenic variants of interleukin 5 (IL5) and tumour necrosis factor alpha (TNF, TNFα). The variants are, besides from being immunogenic in the autologous host, also highly similar to the native 3D structure of the proteins from which they are derived. Certain variants are monomeric mimics of the multimers, where peptide linkers (inert or T helper epitope containing) ensure a spatial organisation of the monomomer units that facilitate correct folding. A subset of variants are monomer TNFα variants that exhibit a superior capability of assembling into multimers with a high structural similarity to the native protein. Also disclosed are methods of treatment and production of the variants as well as DNA fragments, vectors, and host cells.

INTERNATIONAL SEARCH REPORT

ational Application No
PCT/DK 02/00764

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/54 C07K14/525 C07K19/00 A61K39/00 C12N15/62
//A61P37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K C12N A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 00 65058 A (KLYSNER STEEN ;M & E BIOTECH AS (DK)) 2 November 2000 (2000-11-02) page 25 -page 33 page 56 -page 57; figure 5 examples 4,10,11,18 the whole document	1-20, 24-32, 34-36, 38-47
X		1-7,10, 12-14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 June 2003	27. 08. 2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Carl-Olof Gustafsson

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 70245 A (ARIMILLI SUBHASHINI ;CORIXA CORP (US); ZHU SHIRLEY (US); CARTER DA) 27 September 2001 (2001-09-27) page 11, line 11 - line 21 page 12, line 11 -page 15	1-7, 10-15
Y	figures 7-10	1-20, 24-32, 34-36, 38-47

X	WO 99 42597 A (HARVARD COLLEGE) 26 August 1999 (1999-08-26) page 5 page 26, line 10 - line 16 page 29 -page 31 page 52 -page 53	1-7,9-13
Y		1-20, 24-32, 34-36, 38-47

Y	WO 95 05849 A (ELSNER HENRIK ;MOURITSEN & ELSNER A S (DK); MOURITSEN SOEREN (DK)) 2 March 1995 (1995-03-02) page 6 -page 7; examples 3,4,6	16-20

A	EP 0 659 438 A (BOEHRINGER MANNHEIM GMBH) 28 June 1995 (1995-06-28) page 10, line 5 - line 35	1-20, 24-27, 42-47

Y	JUN LI ET AL: "Single chain human interleukin 5 and its asymmetric mutagenesis for mapping receptor binding sites." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 4, 26 January 1996 (1996-01-26), pages 1817-1820, XP002902898 the whole document	16-20

A	JUN LI ET AL: "Mutants of single chain interleukin 5 show asymmetric recruitment of receptor alpha and beta c subunits." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 49, 6 December 1996 (1996-12-06), pages 31729-31734, XP002902899 the whole document	16-20

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHANG H-C ET AL: "A GENERAL METHOD FOR FACILITATING HETERODIMERIC PAIRING BETWEEN TWO PROTEINS: APPLICATION TO EXPRESSION OFALPHA AND BETA T-CELL RECEPTOR EXTRACELLULAR SEGMENTS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 91, November 1994 (1994-11), pages 11408-11412, XP002936749 ISSN: 0027-8424 page 11408 -page 11409 page 11412 ---	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 02/00764

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 27B-41
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 1-18, 21-22, 24-26, 27B-47
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-16 (partialy), 17-20, 24-27a (partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 27B-41

Claims 27b-41 relate to methods of treatment of the human or animal body by surgery or by therapy or diagnostic methods practised on the human or animal body (Rule 39.1(iv)). Nevertheless, a search has been executed for these claims to the extent it was possible when applying the above restrictions of the search. The search has been based on the alleged effects of the compounds or compositions.

Continuation of Box I.2

Claims Nos.: 1-18, 21-22, 24-26, 27B-47

Present claims 1-13, 14-15 and 16-18, 21-22, 24-26, 27b-47 (partially) relate to an extremely large number of possible protein constructs or compositions and related methods due to the rather broad or vague wordings " polymeric protein.... consisting of at least 2 monomeric units " and "one MHC Class II binding amino acid sequence ". Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds, products/compositions and methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the seach has been carried out for those parts of the claims which appear to be supported and disclosed and within invention 1, namely those parts that relate to IL5 constructs comprising " MHC Class II binding amino acid sequences " selected from the ones revealed in claim 14 and sequences claimed.

Further, the search has covered the general aspects of the invention to some extent, although it lacks the necessary precision in the definition of the subject matter. Consequently, the search for the general concept of " MHC Class II binding peptides " and " polymeric protein... consisting of at least 2 monomeric units " will retrieve a pertinent document only if this concept is described in general terms in a reference. Specific solutions previously known and falling under the general concept- but failing to mention this fact- are likely not to be revealed in such a search.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-16 (partialy) and 17-20 and 24-27a (partially)

   Immunogenic analogues of IL5 and method for production, nucleic acid fragments and compositions comprising monomeric units joined via peptide bonds and including at least one MHC Class II binding amino acid sequence and being produced as single chain soluble recombinant products.

2. Claims: 1-16 (partially) and 21/22-27a, 42-47 (partially).

   Dimeric (trimeric....) immunogenic analogues of a human TNF constructs, method for production, nucleic acid fragments and compositions comprising a monomer TNF alpha unit modified to include at least one foreign MHC Class II binding amino acid sequence and being produced as single chain soluble recombinant products.

3. Claims: 22 and 23-27a (partially).

   Monomeric immunogenic analogues of TNF alpha constructs, method for production, nucleic acid fragments and compositions comprising at least one MHC Class II binding amino acid sequence and being produced as single chain soluble recombinant products.
   ** as far as not covered by invention 2 **.

4. Claims: 22-27a, 42-47 (partially).

   Immunogenic analogues of a human TNF constructs, method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified to include at least one foreign MHC Class II binding amino acid sequence into flexible loop 3.
   ** as far as not covered by invention 3 **.

5. Claims: 22-27a, 42-47 (partially).

   Immunogenic analogues of a human TNF constructs, method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified to include at least one foreign MHC Class II binding amino acid sequence and having at least one added monomer stabilising disulfide bridge.
   ** as far as not covered by inventions 3 or 4 **.

6. Claims: 22-27a, 42-47 (partially).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Immunogenic analogues of a human TNF constructs and method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified to include at least one foreign MHC Class II binding amino acid sequence wherein one or more of the N-terminal amino acids 1-9 have been deleted.
** as far as not covered by inventions 3-5 **.

7. Claims: 16, 22-27a, 42-47 (partially).

Immunogenic analogues of a human TNF constructs and method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified to include at least one foreign MHC Class II binding amino acid sequence into loop 1 in an intron position.
** as far as not covered by inventions 3 or 5 or 6 **.

8. Claims: 16, 22-27a, 42-47 (partially).

Immunogenic analogues of a human TNF constructs and method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified to include at least one foreign MHC Class II binding amino acid sequence is introduced as part of an artificial stalk region in the N terminus of human TNF alpha.
** as far as not covered by inventions 3 or 5 or 6 **.

9. Claims: 16, 22-27a, 42-47 (partially).

Immunogenic analogues of a human TNF constructs, method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified to include at least one foreign MHC Class II binding amino acid sequence "so as to stabilize the monomer structure by increasing hydrophobicity".
** as far as not covered by inventions 3-8 **.

10. Claims: 16, 22-27a, 42-47 (partially).

Immunogenic analogues of a human TNF monomer constructs, method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified to include at least one foreign MHC Class II binding amino acid sequence flanked by glycine residues.
** as far as not covered by inventions 3-9 **.

11. Claims: 16, 22-27a, 42-47 (partially).

Immunogenic analogues of a human TNF monomer constructs, method for production, nucleic acid fragments and

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

compositions comprising a monomeric TNF alpha unit modified to include at least one MHC Class II binding amino acid sequence inserted or in-substituted in the D-E loop.
** as far as not covered by inventions 3 or 5 ,6 or 9 or 10**.

12. Claims: 16, 22-27a, 42-47 (partially).

Immunogenic analogues of a human TNF constructs, method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified to include at least one MHC Class II binding amino acid sequence inserted or in-substituted between two identical sub-sequences of human TNF alpha.
** as far as not covered by invention 2 **.

13. Claims: 16, 22-27a, 42-47 (partially).

Immunogenic analogues of a human TNF constructs, method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified to include at least one salt bridge being strengthened or substituted with a disulfide bridge.
** as far as not covered by inventions 5, 6, 9 or 10 **.

14. Claims: 16, 22-27a, 42-47 (partially).

Immunogenic analogues of a human TNF constructs, method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified by introducing mutations that provides a mimic of murine TNF alpha crystalline structure.
** as far as not covered by inventions 3-13 **.

15. Claims: 16, 22-27a, 42-47 (partially).

Immunogenic analogues of a human TNF constructs, method for production, nucleic acid fragments and compositions comprising a monomeric TNF alpha unit modified by at least on point mutation to reduce or abolish potential toxicity.
** as far as not covered by inventions 3-14 **.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/DK 02/00764

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